

Volume - 9

No. 11

November-2016

ISSN: 0972-8988

EISSN: 2231-0916



NAAS Rating : 5.1

Indexed in ESCI-Thomson Reuters, PubMed,  
PubMed Central, DOAJ, Scopus, CABI, CAS etc.

---

# Veterinary World

Open access and peer reviewed journal

---



**Editorial office**

Veterinary World,  
Star, Gulshan Park,  
NH-8A, Chandrapur Road,  
Wankaner - 363621,  
Dist. Morbi, Gujarat, India  
Website: [www.veterinaryworld.org](http://www.veterinaryworld.org)  
E-mail: [editorveterinaryworld@gmail.com](mailto:editorveterinaryworld@gmail.com)



# Veterinary World

**Editorial Office:** Veterinary World, Star, Gulshan Park, NH-8A, Chandrapur Road, Wankaner - 363621, Dist. Morbi, Gujarat, India

ISSN: 0972-8988, EISSN: 2231-0916, [www.veterinaryworld.org](http://www.veterinaryworld.org)

## Editor-in-Chief

Anjum V. Sherasiya - Ex-Veterinary Officer, Department of Animal Husbandry, Gujarat State, India

## Associate Editors

Shambhunath Choudhary - Department of Biomedical & Diagnostic Sciences, College of Veterinary Medicine, The University of Tennessee, 2407 River Drive, Room A 201, Knoxville, TN 37996, U.S.A.

Suresh H. Basagoudanavar - FMD Vaccine Research Laboratory, IVRI, Bangalore - 560024, Karnataka, India

## Editorial board

R. G. Jani - Ex-Coordinator Wildlife Health, Western Region Centre, Indo-US Project, Department of Veterinary Medicine, Veterinary College, Anand - 389001, Gujarat, India

G. N. Gongal - Technical Officer, WHO South-East Asia Regional Office, New Delhi - 110002, India

Ranganath Mamidi - Dr. Julian E Stelzer's Lab, Department of Physiology & Biophysics, Medical School, Case Western Reserve University, Cleveland, OH - 44106, U.S.A.

Md. Tanvir Rahman - Department of Microbiology and Hygiene, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

Deepmala Agarwal - Cancer Prevention Laboratory, Pennington Biomedical Research Center, Baton Rouge, LA, U.S.A.

Foud Kasim Mohammad - Professor, Department of Pharmacology & Toxicology, Vice President for Administrative & Financial Affairs, University of Mosul, P.O. Box 11136, Mosul, Iraq

Abdel-Baset Nasr Sayed Ahmed - Professor and Head, Department of Animal Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt

Nicole Borel - Department of Pathology, Vetsuisse Faculty, University of Zurich, CH-8057 Zurich, Switzerland

B. A. Lubisi - Virology, MED Programme, ARC - Onderstepoort Veterinary Institute, No. 100 Old Soutpan Road, Onderstepoort, Tshwane, 0110, South Africa

Kumar Venkitanarayan - Associate Professor, Graduate Programs Chair, Honors and Pre-Vet Programs Advisor, Department of Animal Science, University of Connecticut, Storrs, CT 06269, U.S.A.

Kemin Xu - Department of Veterinary Medicine, University of Maryland, College Park College Park, MD, 20742, U.S.A.

Vassilis Papatziros - Faculty of Veterinary Medicine, Department of Medicine (Porcine Medicine), University of Thessaly, Thessaly, Greece

Mathias Devreese - Laboratory of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ghent University, Belgium

Sumet Sharma - Edmonton North Animal Hospital, Edmonton, Alberta, T5X3Y7, Canada

K. P. Singh - School of Medicine and Dentistry, University of Rochester, Department of Environmental Medicine, Room: 4-6820, 601 Elmwood Avenue, Box-EHSC, Rochester, New York-14620, U.S.A.

Raj Mohan Raja Muthiah - Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, U.S.A.

Ashok K. Chockalingam - Division of Applied Regulatory Science, U.S. Food and Drug Administration, 10903, New Hampshire Avenue, Silver Spring, Maryland 20993, U.S.A.

Ashutosh Wadhwa - Poxvirus and Rabies Branch, Division of High-Consequence Pathogens and Pathology, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, 1600 Clifton Road, NE, Mail Stop G33, Atlanta, GA 30333, U.S.A.

Luiz Otavio Pereira Carvalho - Laboratory of Immunomodulation and Protozoology, Oswaldo Cruz Institute, Ministry of Health (Brazil), Pavilhão "108" - Sala: 09, Av. Brasil, 4365 - Manguinhos, Rio de Janeiro - RJ, CEP: 21040-360, Brazil.

Editorial office contact person: Anjum V. Sherasiya, email: [editorveterinaryworld@gmail.com](mailto:editorveterinaryworld@gmail.com)

**Ratings of Veterinary World:** NAAS - National Academy of Agricultural Sciences - 5.10, Scimago Journal Rank - 0.265, Impact per publication - 0.47, SNIP - Source Normalized Impact per Paper - 0.600

## Indexing and abstracting

Academic Journals Database, AGORA, AGRICOLA, AGRIS, CABI, CAS, DOAJ, EBSCO, ESCI - Thomson Reuters, Gale, Google Scholar, HINARI, Index Scholar, Indian Animal Science Abstracts, Indian Science Abstracts, JournalSeek, Open J-gate, ProQuest, PubMed, PubMed Central, SCOPUS, TEEAL

## Publisher: Veterinary World

Veterinary World is an open access journal, each issue available free of cost at [www.veterinaryworld.org](http://www.veterinaryworld.org).

We accept online submission only. For more information regarding submission and publication charges, please visit [www.veterinaryworld.org](http://www.veterinaryworld.org)

Printed and Published by Dr. Anjum V. Sherasiya on behalf of Veterinary World. Printed and Published at Star, Gulshan Park, N.H. 8A, Chandrapur Road, Wankaner-363621, Dist. Morbi, Gujarat, India.  
Editor-in-Chief: Dr. Anjum V. Sherasiya

---

# Veterinary World

---

ISSN: 0972-8988, EISSN: 2231-0916, [www.veterinaryworld.org](http://www.veterinaryworld.org)

---

**Volume-9**

**No.11**

**November-2016**

---

The articles in Veterinary World are open access articles licensed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.

---

Review (Published online: 02-11-2016)

1. Relationship of prepartum udder and teat measurements with subsequent milk production traits in primiparous Nili-Ravi buffaloes - T. Chandrasekar, Kalyan Sundar Das, Showkat A. Bhat, J. K. Singh, Thulasiraman Parkunanan, K. Puhle Japheth, Mayur R. Thul and Pranay Bharti  
Veterinary World, 9(11): 1173-1177

Research (Published online: 02-11-2016)

2. Clinico-anesthetic changes following administration of propofol alone and in combination of meperidine and pentazocine lactate in dogs - A. K. Anandmay, L. L. Dass, A. K. Sharma, M. K. Gupta, K. K. Singh and B. K. Roy  
Veterinary World, 9(11): 1178-1183

Research (Published online: 03-11-2016)

3. Enterohemorrhagic *Escherichia coli* O157 in milk and dairy products from Libya: Isolation and molecular identification by partial sequencing of 16S rDNA - Aboubaker M. Garbaj, Enas M. Awad, Salah M. Azwai, Said K. Abolghait, Hesham T. Naas, Ashraf A. Moawad, Fatim T. Gammoudi, Ilaria Barbieri and Ibrahim M. Eldaghayes  
Veterinary World, 9(11): 1184-1189

Review (Published online: 04-11-2016)

4. Anaplasma species of veterinary importance in Japan - Adrian Patalinghug Ybañez and Hisashi Inokuma  
Veterinary World, 9(11): 1190-1196

Research (Published online: 05-11-2016)

5. Influence of drinking water containing Aloe vera (*Aloe barbadensis* Miller) gel on growth performance, intestinal microflora, and humoral immune responses of broilers - Meisam Shokraneh, Gholamreza Ghalamkari, Majid Toghyani and Nasir Landy  
Veterinary World, 9(11): 1197-1203

Research (Published online: 08-11-2016)

6. Acute phase response in lame crossbred dairy cattle - A. Bagga, Swaran Singh Randhawa, S. Sharma and B. K. Bansal  
Veterinary World, 9(11): 1204-1208

Research (Published online: 08-11-2016)

7. In vitro evaluation of different varieties of maize fodder for their methane generation potential and digestibility with goat rumen liquor - Shalini Vaswani, Ravindra Kumar, Vinod Kumar, Debashis Roy and Muneendra Kumar  
Veterinary World, 9(11): 1209-1213

Research (Published online: 09-11-2016)

8. Prevalence and burden of gastrointestinal parasites in cattle and buffaloes in Jabalpur, India - Priyanka Marskole, Yamini Verma, Alok Kumar Dixit and Madhu Swamy  
Veterinary World, 9(11): 1214-1217

Research (Published online: 10-11-2016)

9. Biocomputational analysis of evolutionary relationship between toll-like receptor and nucleotide-binding oligomerization domain-like receptors genes - Rabia Bhardwaj, Chandra Shekhar Mukhopadhyay, Dipak Deka, Ramneek Verma, P. P. Dubey and J. S. Arora  
Veterinary World, 9(11): 1218-1228

Review (Published online: 11-11-2016)

10. Laboratory animal models for esophageal cancer - Dhanya Venugopalan Nair and A. Gopala Reddy  
Veterinary World, 9(11): 1229-1232

Research (Published online: 12-11-2016)

11. Evaluation of five treatments to control intestinal parasites in sheep in Ayapango, state of Mexico - Rafael Heredia, Emma Aguilar, Camilo Romero, Linda Bautista and Germán Mendoza  
Veterinary World, 9(11): 1233-1237

Research (Published online: 14-11-2016)

12. Relationship between hepcidin and oxidant/antioxidant status in calves with suspected neonatal septicemia - E. E. Erkilic, H. M. Erdogan, M. Ogun, A. H. Kirmizigul, E. Gokce, M. Kuru and A. Kukurt  
Veterinary World, 9(11): 1238-1241

---

Research (Published online: 14-11-2016)

13. Prevalence of ketosis in dairy cows in milk shed areas of Odisha state, India - Sangram Biswal, Dhruba Charan Nayak and Kautuk Kumar Sardar  
Veterinary World, 9(11): 1242-1247

Review (Published online: 15-11-2016)

14. Helminth infections in domestic dogs from Russia - T. V. Moskvina and A. V. Ermolenko  
Veterinary World, 9(11): 1248-1258

Research (Published online: 16-11-2016)

15. Study of antimicrobial resistance due to extended spectrum betalactamase-producing *Escherichia coli* in healthy broilers of Jabalpur - Arpita Shrivastav, R. K. Sharma, Y. P. Sahni, Neeraj Shrivastav, Vidhi Gautam and Sachin Jain  
Veterinary World, 9(11): 1259-1263

Research (Published online: 17-11-2016)

16. Inhibition of bovine platelets aggregation in response to Hyalomma anatolicum salivary gland proteins/peptides - Surbhi, Nirmal Sangwan, Arun K. Sangwan, Vijender Singh and Ankit Kumar  
Veterinary World, 9(11): 1264-1268

Research (Published online: 17-11-2016)

✓ 17. Benefits of pomegranate (*Punica granatum* Linn) fruit extracts to weight changes, total protein, and uric acid in white rats (*Rattus norvegicus*) as an animal model of acute renal failure - Hardany Primarizky, Wiwik Misaco Yuniarti and Bambang Sektiari Lukiswanto  
Veterinary World, 9(11): 1269-1274

Research (Published online: 18-11-2016)

18. Comparison of veterinary health services expectations and perceptions between oncologic pet owners, non-oncologic pet owners and veterinary staff using the SERVQUAL methodology - Hugo Gregório, Patricia Santos, Isabel Pires, Justina Prada and Felisbina Luísa Queiroga  
Veterinary World, 9(11): 1275-1281

Research (Published online: 21-11-2016)

19. Chronic exposure to indoxacarb and pulmonary expression of toll-like receptor-9 in mice - Sandeep Kaur, C. S. Mukhopadhyay and R. S. Sethi  
Veterinary World, 9(11): 1282-1286

Research (Published online: 23-11-2016)

20. Value added by *Spirulina platensis* in two different diets on growth performance, gut microbiota, and meat quality of Japanese quails - Mohamed S. Yusuf, Marwa A. Hassan, Mohamed M. Abdel-Daim, Adel S. El nabtiti, Ali Meawad Ahmed, Sherief A. Moawed, Ahmed Kamel El-Sayed and Hengmi Cui  
Veterinary World, 9(11): 1287-1293

Research (Published online: 25-11-2016)

21. Allelic and genotypic frequencies in polymorphic Booroola fecundity gene and their association with multiple birth and postnatal growth in Chhotanagpuri sheep - Thanesh Oraon, D. K. Singh, Mayukh Ghosh, S. S. Kullu, Rajesh Kumar and L. B. Singh  
Veterinary World, 9(11): 1294-1299

Research (Published online: 27-11-2016)

22. Seroprevalence of Rotavirus infection in pig population of Arunachal Pradesh - G. B. Garam, D. P. Bora, B. Borah, M. Bora and S. K. Das  
Veterinary World, 9(11): 1300-1304

Research (Published online: 27-11-2016)

23. Prophylactic effect of *Nigella sativa* against lead acetate induced changes in spermiogram, reproductive hormones and gonadal histology of rats - Mohammed Abdulrazzaq Assi, Mohammed Noor Mohd Hezmee, Yusuf Abba, Md Sabri Md Yusof, Abd Wahid Haron, Mohamed Ali Rajion and Mashaan Abbas Al-Zuhairi  
Veterinary World, 9(11): 1305-1311

Research (Published online: 28-11-2016)

24. Occurrence and antimicrobial resistance of pathogenic *Escherichia coli* and *Salmonella* spp. in retail raw table eggs sold for human consumption in Enugu state, Nigeria - O. Josephine Okorie-Kanu, E. Vivienne Ezenduka, C. Onwuchokwe Okorie-Kanu, L. Chinweokwu Ugwu and U. John Nnamani  
Veterinary World, 9(11): 1312-1319

Research (Published online: 29-11-2016)

25. Steroid and metabolic hormonal profile of porcine serum vis-à-vis ovarian follicular fluid - Soumen Naskar, S. Borah, Y. Vashi, R. Thomas, D. K. Sarma, J. Goswami and S. K. Dhara  
Veterinary World, 9(11): 1320-1323

Research (Published online: 29-11-2016)

26. Honeybee product therapeutic as stem cells homing for ovary failure - Erma Safitri, Thomas V. Widiyatno and R. Heru Prasetyo  
Veterinary World, 9(11): 1324-1330

Research (Published online: 30-11-2016)

27. Amelioration of Gamma-hexachlorocyclohexane (Lindane) induced renal toxicity by *Camellia sinensis* in Wistar rats - W. L. N. V. Vara Prasad, Ch. Srilatha, N. Sailaja, N. K. B. Raju and N. Jayasree  
Veterinary World, 9(11): 1331-1337

\*\*\*\*\*



## Benefits of pomegranate (*Punica granatum* Linn) fruit extracts to weight changes, total protein, and uric acid in white rats (*Rattus norvegicus*) as an animal model of acute renal failure

Hardany Primarizky, Wiwik Misaco Yuniarti and Bambang Sektiari Lukiswanto

Department of Veterinary Clinic, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia.

**Corresponding author:** Hardany Primarizky, e-mail: [kikken.zeyra@gmail.com](mailto:kikken.zeyra@gmail.com),  
WMY: [wiwikmisaco@yahoo.com](mailto:wiwikmisaco@yahoo.com), BSL: [bamsekti@yahoo.com](mailto:bamsekti@yahoo.com)

**Received:** 21-03-2016, **Accepted:** 13-10-2016, **Published online:** 17-11-2016

**doi:** 10.14202/vetworld.2016.1269-1274 **How to cite this article:** Primarizky H, Yuniarti WM, Lukiswanto BS (2016) Benefits of pomegranate (*Punica granatum* Linn) fruit extracts to weight changes, total protein, and uric acid in white rats (*Rattus norvegicus*) as an animal model of acute renal failure, *Veterinary World*, 9(11): 1269-1274.

### Abstract

**Aim:** The occurrence of acute renal failure (ARF) cases continues to increase every year. Some of the cases are due to nephrotoxic effect caused by overdose of antibiotic consumption or abuse of the drug, gentamicin. An antibiotic therapy that can be used to overcome in such a case is the pomegranate extracts. However, until now, studies using pomegranate for cases of ARF have not been done. This study aims to determine changes in body weight, the levels of total protein (TP), and the levels of serum uric acid (UA) as a result of the pomegranate extract consumption.

**Materials and Methods:** A total number of 32 rats (*Rattus norvegicus*) were divided into four groups randomly. One group was assigned as the control group (P0) and given intraperitoneal (i.p.) saline and 0.3% carboxy methyl cellulose sodium (CMC) Na; P1 was provided with 80 mg/kg bw/i.p. gentamicin and 0.3% CMC Na orally, P2 was supplied with 80 mg/kg bw/i.p. gentamicin and ellagic acid in 0.3% CMC Na, and P3 was given 80 mg/kg bw/i.p. gentamicin and 150 mg/kg bw pomegranate extract in 0.3% CMC Na. The provision of treatment was carried out in 8 days and followed by making the overthrow of body weight and blood sampling for the examination of study variables.

**Results:** The results taken by doing the analysis of variance method for the four treatment groups show that the control group (P0) has significant differences from P1, P2, and P3 ( $p < 0.05$ ), but there are no significant differences among the other three treatment groups. Meanwhile, the average values of serum UA levels among P1, P2, and P3 indicate significant differences.

**Conclusion:** In conclusion, the administration of pomegranate extracts in the treatment of nephrotoxicity toward rats is effective to maintain normal body weight, normal TP levels, and the UA blood serum of the rats. As this study is a preventive therapy, it needs further researches about the effective dose as a curative therapy, its level of effectiveness and its long-term side effects.

**Keywords:** acute renal failure, gout, pomegranate extract, total protein.

### Introduction

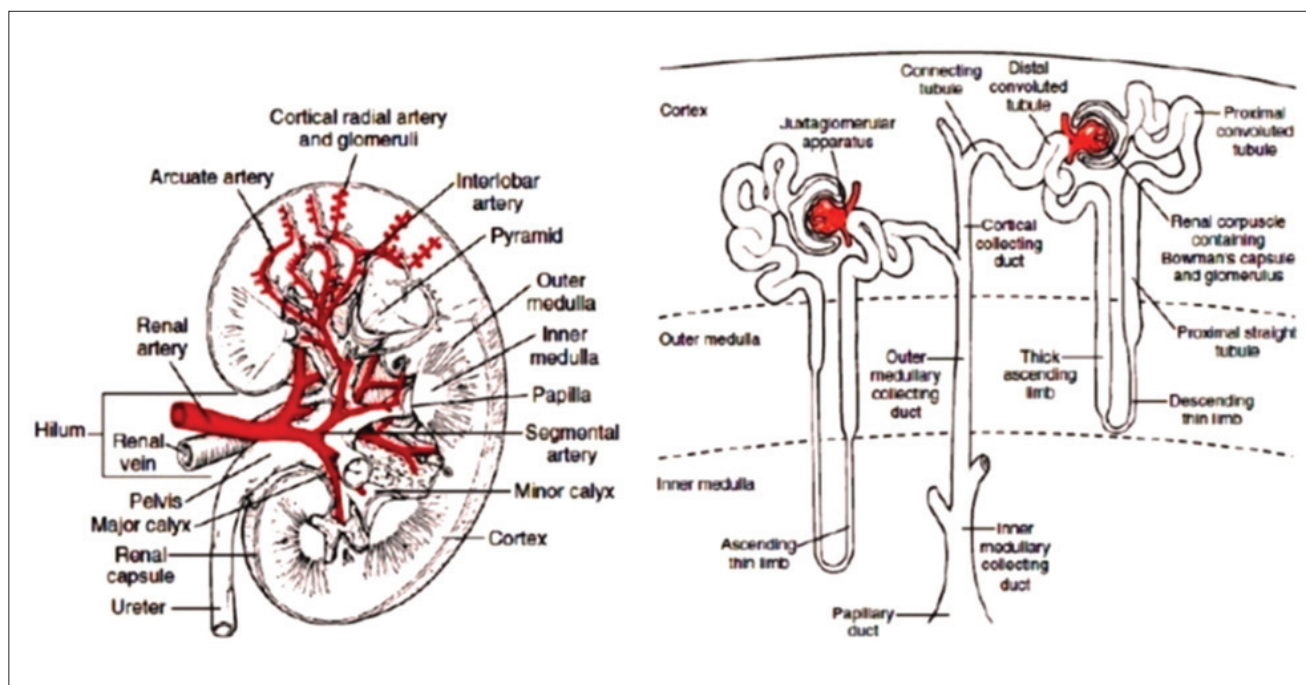
Renal failure is the failure of kidneys to remove excess metabolites which are accumulated in the blood. It is a systemic disease and a final common pathway of various urinary tract and kidney diseases [1]. It triggers electrolyte balance disorders, acid-base and water, renal failure. The failure is classified into acute renal failure (ARF) and chronic renal failure [2]. The ARF is characterized by the decrease of the urine volume and an increase of urea and creatinine values in 24 h [3]. The progressive weight loss, increased levels of uric acid (UA), and a decrease of total plasma protein are also major indications of patients with ARF.

The number of patients with kidney failure increases quite a lot and is predicted to continue every year. This is due to factors such as false dose of nephrotoxic drug consumption, lack of public awareness about the dangers of kidney disease and its prevention which should be done from an early age, and so on [4]. Determining kidney damage can be carried out by checking physical parameters such as weight measurement and biochemical parameters such as the examination of the levels of urea, creatinine, UA and the total serum protein.

Kidneys are bean-shaped organs that lie behind the peritoneum, on both sides of the vertebral column. The cross-section of the kidney is divided into two parts, namely, the cortex and the medulla in which the cortex is darker than the medulla [5]. The renal medulla is conical masses which are called renal pyramids [6]. The size of kidney in various species is primarily determined by the number of their nephrons [7]. The general structure of histological kidney can be shown in Figure-1.

Most potassium and UA is reabsorbed by a distal convoluted tubule and secreted into a distal

Copyright: Primarizky, et al. Open Access. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.



**Figure-1:** Kidney general structure. (a) The general structure of the kidney, (b) components of the nephron and ductus collectivus system [8].

tubule. The formation of ammonia, the acidification of urine, and the water phase setting of the water and acid-base balance ensues in the distal convoluted tubules. The secretion and the selective reabsorption process are completed within the distal convoluted tubules and ductus collectivus [8]. Kidneys have a secretory function, one of which is to excrete end products of nitrogen and protein metabolism (especially urea, UA, and creatinine), foreign chemicals (such as pharmaceuticals), hormones, and other metabolites [9].

Pomegranate fruit is round with the skin colors of green, purple, white, reddish-brown, or purple-black. Its red or white seeds (which are very high in number) are small, slightly flattened, elliptical, and hard [10]. The characteristics of pomegranate can be shown in Figure-2.

One of the components of pomegranate is ellagic acid (EA), an ingredient that can protect cell damage which is caused by free radicals. This capability will synergistically increase when it is combined with another strong antioxidant composition of pomegranate, anthocyanidins [10,12].

The degree of proteinuria and the composition of proteins in the urine depend on the mechanism of kidney injury resulting the loss of protein. A large number of proteins normally passes through the glomerular capillary but does not penetrate the urine. The glomerular wall's substance and selectivity prevent the transport of albumin, globulin, and protein with other large molecular weight to penetrate its walls. If this barrier is broken, there is a plasma protein leakage in the urine (glomerular proteins). The smaller protein (<20 kDa) is freely filtered but reabsorbed by the proximal tubule. The amount of protein that comes

out with urine reduces the levels of the total protein (TP) serum in patients with kidney failures.

UA is a compound of nitrogen produced from catabolism of purines either from diet or endogenous nucleic acids (DNA deoxyribonucleic acid). It is largely excreted through the kidneys and slightly secreted through the gastrointestinal tract. The increasing levels of the UAs called hyperuricemia which can be caused by an excessive production or a decreasing excretion (such as in renal failure). The increasing level of UA in the urine is called uricosuria. The excretion of UA in the urine depends on the levels of UA in the blood, glomerular filtration, and tubular secretion of UA into the urine [13-16].

The results of the previous studies show that 30% of patients who were treated with gentamicin for more than 7 days indicated signs of nephrotoxicity. The gentamicin nephrotoxicity is one of the most common causes of the ARF [17]. Gentamicin belongs to a class of aminoglycosides and is an antibiotic commonly used to treat Gram-negative bacterial infections in humans and animals. It can cause nephrotoxicity and is a way to get an animal model of the ARF. The intraperitoneal (i.p.) administration of gentamicin leads to an increasing formation of superoxide to cause oxidative stress and cellular damage in the proximal renal tubules of the kidney. If it is prolonged, it will cause an ARF [18].

Reactive oxygen species are potential mediators involved in the gentamicin induction of renal impairment. The gentamicin leads to an increasing superoxide anions, peroxynitrite anions, and hydrogen peroxide from the renal cortex mitochondria. A raise of nitric oxide which occurs by the activation of the inducible nitric oxide synthase has proven to cause



**Figure-2:** *Punica granatum* L. [11].

kidney disorders through several mechanisms. The gentamicin also produces interstitial edema and epithelial necrosis [19].

Currently, there have been many attempts to find cheap and safe alternative medicine for the treatments of the kidney for instance using materials derived from plants. One of the plants whose benefits have been researched is pomegranate. It is a fruit which contains many phenolic compounds, namely, EA and punicalagin. In addition, the fruit is also composed of anthocyanin compounds such as prodelfinidin, delphinidin, sianidin, and pelargonidin [12]. However, until now the use of pomegranate to prevent the ARF has not been done. There are advantages of using herbal medicine such as it is easily produced, its application is simple, and its cost is cheaper than the cost of pharmaceutical drugs. In general, the use of herbal medicine to cure diseases takes a long time, but the effect is to give protection, build and imply positively for other organs. This is different from consuming chemical drugs which have a faster-working process but damage both infected and normal organs [20].

## Materials and Methods

### Ethical approval

This study was duly approved by Institutional Animal Ethics Committee.

### Experimental design

This study is an experimental laboratory research. The research design is a randomized control group - only post-test design. The samples and the treatments were under scalable controlled conditions to keep the effects more valid. The production of animal models and its maintenance were performed in the Laboratory of Biochemistry, Faculty of Medicine, Universitas Airlangga, while the examination of various variables of the study was conducted at the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Universitas Airlangga. This study was conducted after obtaining a certificate of conduct issued by a research ethics committee.

### Research materials

The experimental units in this study were male strain Wistar white rats (*Rattus norvegicus*) which were obtained from the Animal Care Unit Experiment Universitas Gajah Mada, Yogyakarta. White rats were

used because they were inexpensive, easily obtained, and maintained. The rats used in this study should have been male with the criteria of homogeneous samples, the age was 2.5 months old, the weight was between 150 and 200 g, and they were in good health condition, which was characterized by shiny fur and eyes, and agile movement.

The tools used in this study were 1 cc syringes, a 3-cc syringe and a 10-cc syringe, animal feed and drink containers, husks for the base of the cattle pen, a size 8 feeding tube, a mortar, cotton, tweezers, and a scale. This study used standardized pomegranate extracts which contained 40% EA and pure EA produced by Xi'an Biof Bio-Technology Co., Ltd., gentamicin, 0.3% carboxy methylcellulose (CMC) Na, and 70% alcohol. The samples used were the weight and the 3 ml blood injected out of 32 male rats' blood which was used for the examination of the TP content and the serum UA.

### Research methods

The standardized pomegranate extracts given to the experimental animals were suspended with 0.3% CMC Na in the mortar to keep the homogeneity of the solvents [21]. The preparations were also made before be given to the experimental animals. The production of 0.3% CMC Na was done by sprinkling 0.3 g CMC Na in 100 ml of warm distilled water.

This study used 32 male rats Wistar aged 2.5 months whose weight was between 150 and 200 g. Having adapted for 1 week, they were divided into four groups, namely, P0, P1, P2, and P3, in which each group was treated in eight cycles. The control group (P0) was given i.p. saline and 0.3% CMC Na orally, P1 was provided with 80 mg/kg bw/i.p. gentamicin and 0.3% oral CMC Na, P2 was parted with 80 mg/kg bw/i.p. gentamicin and 60 mg/kg bw EA in 0.3% CMC Na per oral, and P3 was given 80 mg/kg bw/i.p. gentamicin and pomegranate extracts at a dose of 150 mg/kg bw in 0.3% Na CMC orally. The volume of saline and gentamicin was 0.4 cc, while the volume of the CMC Na solvent, EA and pomegranate extracts was 2 cc. After 8 days of treatment, the weighing and the sampling of intracardiac blood was done. A dose of EA which was administered for 8 days was 60 mg/kg bw/po/day. Based on the content of 40% EA found in the extracts, the fruit extract dose was 150 mg/kg/dd/po/day for 8 days [22]. Weighing had been done before performing blood sampling. The intracardiac blood samples were taken after the white rats were anesthetized with ether incision in the thoracic region. Blood was collected as much as possible for an examination of the TP content and the serum UA.

The production and checking of the levels of serum TP were conducted by using the Biuret method. Based on the method, the principles of the protein determination levels in serum was the measurement of the purple complex light absorption of proteins to reacting with a biuret reagent. The complex was formed by



proteins with  $\text{Cu}^{2+}$  ions in a biuret reagent under alkaline conditions. The higher the intensity of the absorbed light by the tool meant the higher the protein content was in the serum. The serum UA examination was done by the enzymatic method. The principle of the checking UA levels in enzymatic method was that uricase broke down the UA into allantoin and hydrogen peroxide. Then the presence of peroxidase, peroxide, N-ethyl-N-(2-hydroxy-3-sulfo-propyl)-3-methylaniline (TOOS) and 4-aminophenazone formed the quinoneimine color. The intensity of the formed red color was proportional with the concentration of UA [23].

The results of the study were presented in a table of the average value and the standard deviation (SD). The treatment effects of the research variables were determined by performing a statistical analysis of variance (ANOVA). They were considered significant if  $F \text{ count} > F \text{ table}$  or  $p < 0.05$ . Otherwise, the least significant difference test was proceeded.

## Results

Weighing the white rats was firstly done to examine the variables of the study. The results are shown in Table-1.

The research variables are the levels of TP and UA from the blood serum which is taken from all of the rats after 8 days of treatment. The test results of the TP and UA in the Laboratory of Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Universitas Airlangga to 32 rats which were divided into four treatments and eight cycles, namely P0 by administering saline and CMC Na as a control, P1 by administering gentamicin and CMC Na, P2 by administering gentamicin, CMC Na and EA, and P3 by administering gentamicin, CMC Na and pomegranate extract, were processed by applying SPSS 17 for Windows by using ANOVA. The results of blood serum levels of TP and acid veins are shown in Table-2.

The average values and the SD of the TP levels in groups P0, P1, P2, and P3 are  $6.367 \pm 0.3615$ ,  $0.3615 \pm 5.933$ ,  $5.917 \pm 0.5913$ ,  $5.683 \pm 0.3312$ , respectively. Results of the statistical calculation of the total blood serum proteins are displayed in Table-3.

The results of the study using ANOVA for the four treatment groups display that the control group

(P0) shows a significant difference from the treatment groups (P1, P2, and P3), and there is no significant difference among them. The average values of serum UA levels in groups P0, P1, P2, and P3 are  $1.583 \pm 0.1472$ ,  $1.450 \pm 0.6156$ ,  $1.8468 \pm 1.933$ ,  $1.300 \pm 0.1673$ , respectively. The statistical calculation results of the serum UA levels in the blood are shown in Table-4.

The results reveal that the control group (P0) shows highly significant differences from the treatment groups (P1, P2, and P3), but important distinctions are found among the three groups while a highly important discrepancy is gathered between the treatment groups P2 and P3.

## Discussion

The study of blood serum TPs in white rats between the control group (P0) and the treatment groups (P1, P2, and P3) demonstrates significant differences ( $p < 0.05$ ). P1 displays the highest levels of TPs because this group of white experimental rats with gentamicin was given 0.3% CMC Na. The decreasing levels of TP reflect a decline of the protein amount in the blood due to the gentamicin which has already penetrated into the kidney cells, especially the epithelial cells of the proximal tubule. This causes malfunction, impaired metabolism of the membrane intracellular, and damage of the epithelial cells of proximal tubular kidney which eventually lead to the ARF [24]. The damage of the glomerulus causes the glomerular selectivity walls fail to prevent proteins with large molecular weight to be out of the urine. It induces the leakage of plasma proteins which leads to urine and generates a condition of proteinuria [25].

The serum levels of TPs in white rats by the administration of gentamicin and EA (P2) suffers insignificant reduction than the treatment group P1. It can be noted that there are decreasing levels of the total blood serum proteins which are caused by the improved kidney cells. Meanwhile, the levels of TP blood serum of the white rats by the administration of gentamicin and pomegranate extracts in (P3) sustain inessential degradation than the treatment of group P2. P2 gets the treatment with the best results to reduce the levels of the TPs even though the outcome of the four treatment groups is still within the normal

**Table-1:** Results of the white rats' body weight measurement (g) before and after the treatments.

Cycles	Weight (g) P0		Weight (g) P1		Weight (g) P2		Weight (g) P3	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
1	170	195	160	190	175	200	200	200
2	190	200	150	160	170	220	195	220
3	170	190	170	160	155	130	180	180
4	160	190	180	150	170	190	190	205
5	170	175	170	190	190	220	200	240
6	155	175	200	170	150	160	200	220
7	200	225	175	170	180	210	150	150
8	185	215	150	175	200	190	180	180



**Table-2:** Results of the white rat blood serum examination in the form of TP and UA.

Cycles	P0		P1		P2		P3	
	TP	UA	TP	UA	TP	UA	TP	UA
1	6.4	1.5	5.5	1.1	5.5	1.3	5.4	1.2
2	6.2	1.5	6.5	2.7	5.7	1.2	5.3	1.1
3	5.8	1.4	6.2	1.3	5.8	1.1	5.5	1.5
4	6.4	1.8	5.9	1.2	5.6	1.2	6.1	1.2
5	6.9	1.7	5.8	1.2	5.8	1.1	6.0	1.5
6	6.5	1.6	5.7	1.2	7.1	5.7	5.8	1.3
7	6.3	1.2	6.5	1.3	5.9	1.4	5.2	1.1
8	5.7	1.4	5.5	1.6	5.7	1.4	5.6	1.5

TP=Total protein, UA=Uric acid

**Table-3:** Mean and SD of the white rats' (*R. norvegicus*) total blood serum proteins after treatments.

Treatments	Levels of TP ( $\bar{X} \pm SD$ )
P0	6.367 <sup>a</sup> ±0.3615
P1	5.933 <sup>b</sup> ±0.3615
P2	5.917 <sup>b</sup> ±0.5913
P3	5.683 <sup>b</sup> ±0.3312

Different superscripted signs in the same column indicate significant differences  $P < 0.05$ . *R. norvegicus*=*Rattus norvegicus*, SD=Standard deviation, TP=Total protein

**Table-4:** Average values and SD of the serum UA levels in white rat (*R. norvegicus*) after treatments.

Treatments	UA levels ( $\bar{X} \pm SD$ )
P0	1.583 <sup>a</sup> ±0.1472
P1	1.450 <sup>bc</sup> ±0.6156
P2	1.933 <sup>c</sup> ±1.8468
P3	1.300 <sup>b</sup> ±0.1673

Different superscripted signs in the same column indicate significant differences  $P < 0.05$ . *R. norvegicus*=*Rattus norvegicus*, SD=Standard deviation, UA=Uric acid

values. The normal levels of the total blood serum proteins of male strain Wistar rats range from 5.0 to 8.0 g/dL [25]. It shows that the combination of various active ingredients in pomegranates has a good effect because it can form a synergistic formulation [26].

Pomegranate (*Punica granatum* Linn) in group P3 contains polyphenols as the main ingredient and punicalagin and EA as the active ingredients. The punicalagin in pomegranates has an antioxidant activity up to 89%. It cannot be directly absorbed by the body because it has a lot of molecules which will undergo hydrolysis in the gut before the absorption. The hydrolysis in the intestine is done by the normal microbes in the gut to become EA which exists in the digestive tract [27]. The low concentrations of EA in the plasma are due to its low solubility in water. The insoluble EA metabolism is caused by microflora activities within the digestive tract. The existence of polyphenols in pomegranate extracts could increase the solubility of EA in the digestive tract. Moreover, polyphenols also have an ability to inhibit the metabolism of EA done by intestinal microflora through their antibacterial activity [12].

The UA levels in the control group (P0) indicate a significant difference from the treatment groups (P1, P2, and P3). The results show that the treatment group P2 gives a better effect in decreasing the UA levels than the treatment group P3. This is because EA contains an antioxidant which decreases the levels of blood biochemistry toward normal values when there is a metabolic disorder in the blood [12]. The normal levels of serum UA blood of male rats strain Wistar range from 0.30 to 1.52 mg/dL [16].

## Conclusion and Suggestion

### Conclusion

The administration of pomegranate extracts as a nephrotoxicity treatment in white rats can maintain a normal weight of the rats, and the normal levels of TP and UA blood serum of white rats.

### Suggestion

As this study is a preventive therapy, it is necessary to conduct further researches on the effective dose of the pomegranate extracts as a curative therapy, the level of effectiveness and their long-term side effects.

### Authors' Contribution

WMY has planned and designed the work of the research. HP has conducted the research work, such as collecting samples and doing the laboratory work. BSL has analyzed the data and provided technical supports. The article was prepared under the guidance of WMY and BSL. All authors are participated in making of article's draft, read and approved it.

### Acknowledgments

The authors are very thankful to Faculty of Veterinary Medicine, Faculty of Medicine and Veterinary Teaching Hospital, Universitas Airlangga, Surabaya, for providing all the type of facilities to conduct the research and also to Bantuan Operasional Perguruan Tinggi Negeri (Grant no. 5242/UN3/KR/2013, on April 22<sup>nd</sup> 2013) (State University Operational Fund) for funding this research.

### Competing Interests

The authors declare that they have no competing interests.

### References

- Day, R.A., Beverly, W. and Pauline, P. (2009) Brunner and Suddarth's Textbook of Canadian Medical-Surgical Nursing. 6<sup>th</sup> ed. Lippincott and Wilkins, USA.
- Tambayong, J. (2001) Patofisiologi. Penerbit Buku Kedokteran, ECG, Jakarta.
- Bartges, J. and David, J.P. (2011) Nephrology and Urology of Small Animals. 1<sup>st</sup> ed. Willey-Blackwell, UK.
- Sutardjo, B. and Fransisca, K. (2012) Dialife Magazine: Kidney Health Information Bulletin. June-July Edition 2012.
- Eurel, J.A. and Brian, L.F. (2006) Textbook of Veterinary Histology. 6<sup>th</sup> ed. Blackwell Publishing, Asia.
- Guyton, A.C. and Hall, J.E. (2006) Textbook of Medical Physiology. 11<sup>th</sup> ed. WB Saunders Co., Philadelphia, PA. p859-864.
- Ganong, W.F. (2001) Review of Medical Physiology.

- McGraw-Hill Companies, Inc., San Francisco. p675-678.
8. Junquiera, L.C. and Carneiro, L. (2003) Basic Histology: Text & Atlas. 10<sup>th</sup> ed. The McGraw-Hill Companies Inc., New York.
9. Bijanti, R., Yulianti, M.G.A., Wahjuni, R.S. and Utomo, R.B. (2010) Buku Ajar Patologi Klinik Veteriner. Edisi Pertama. Airlangga University Press, Surabaya.
10. Lansky, E.P. and Newman, R.A. (2007) Review: *Punica granatum* (pomegranate) and its potential for prevention and treatment of inflammation and cancer. *J. Ethnopharmacol.*, 109(2): 177-206.
11. Budka, D. (2008) Active Ingredients, Their Bioavailability and the Health Benefits of the *Punica granatum* Linn (Pomegranate). MSML Research Unit, London.
12. Seeram, N.P., Adam, L.S., Henning, S.M., Niu, Y., Zhang, Y., Nair, M.G. and Heber, D. (2005) *In vitro* anti-proliferative, apoptotic, and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice. *J. Nutr. Biochem.*, 16: 360-367.
13. Koka, R.M., Huang, E. and Lieske, J.C. (2000) Adhesion of uric acid crystals to the surface of renal epithelial cells. *Am. J. Physiol. Renal Physiol.*, 278: F989-F998.
14. Verdecchia, P., Schillaci, G., Reboldi, G.P., Santeusania, F., Porcellati, C. and Brunetti, P. (2000) Relation between serum uric acid and risk of cardiovascular disease in essential hypertension; the PIUMA study. *Hypertension*, 36: 1072-1078.
15. Mazzali, M., Hughes, J., Kim, Y.G., Jefferson, J.A., Kang, D.H., Gordon, K.L., Lan, H.Y., Kivlighn, S. and Johnson, R.J. (2001) Elevated uric acid increases blood pressure in the rat by a novel crystal-independent mechanism. *Hypertension*, 38: 1101-1106.
16. Kang, D.H., Nakagawa, T., Feng, L., Watanabe, S., Han, L., Mazzali, M., Truong, L., Harris, R. and Johnson, R.J. (2002) A role for uric acid in the progression of renal disease. *J. Am. Soc. Nephrol.*, 13: 2888-2897.
17. Singh, A.P., Muthuraman, A., Jaggi, A.S., Singh, N., Grover, K. and Dhawan, R. (2012) Review: Animal models of acute renal failure. *Pharmacol. Rep.*, 64: 31-44.
18. Mahurkar, N., Mumtaz, M. and Ifthekar, S. (2012) Protective effect of aqueous and methanolic extracts of *Lagenaria siceraria* seeds in gentamicin induced nephrotoxicity. *Int. J. Res. Ayurveda Pharm.*, 3(3): 443-446.
19. Avdagic, N., Cosovic, E., Nakas-Icindic, E., Mornjakovic, Z., Zaciragic, A. and Hadzovic-Dzuvo, A. (2008) Spirulina platensis protects against renal injury in rats with gentamicin-induced acute tubular necrosis. *Bosn. J. Basic Med.*, 8(4): 331-336.
20. Soenanto, H. (2005) Musnahkan Penyakit Dengan Tanaman Obat. Puspa Swara, Jakarta.
21. Palanysamy, D., Kannan, S.E. and Bhojraj, S. (2007) Protective and therapeutic effects of the Indian medicinal plant *Pterocarpus santalinus* on D-galactosamine-induced liver damage. *Asian J. Tradit. Med.*, 2(2): 51-57.
22. Ibrahim, M.A.L. and Sayed, A.A.S. (2012) Comparative study of quercetin or/and urate oxidase against gentamicin-induced nephrotoxicity and oxidative stress in rat kidneys. *J. Am. Sci.*, 8(1): 600-607.
23. Roche (2010). Available from: [http://www.roche-applied-science.com/wcsstore/CBCatalogAssetStore/Articles/05837880900\\_03.11.pdf](http://www.roche-applied-science.com/wcsstore/CBCatalogAssetStore/Articles/05837880900_03.11.pdf). Accessed on 07-09-2013.
24. Dalimunthe, A. (2008) Monitoring Effectiveness of Multiple Intravenous Dose of Gentamicin Against Pneumonia Patients Community at Center General Hospital H. Adam Malik. Thesis. University of North Sumatera, Medan. Indonesia.
25. Toblli, J.E., Bevione, P., Di Gennaro, F., Madalena, L., Cao, G. and Angerosa, M. (2012) Review article; understanding the mechanisms of proteinuria: Therapeutic implications. *Int. J. Nephrol.*, 2012: 1-13.
26. Seeram, N.P., Schulman, R.N. and Heber, D. (2006) Pomegranate Ancient Roots to Modern Medicine. 1<sup>st</sup> ed. Taylor and Francis Group, New York. p2-99.
27. Zhang, Y., Wang, D., Lee, R., Henning, S.M. and Heber, D. (2009) Absence of pomegranate ellagitannins in the majority of commercial pomegranate extract: Implications for standardization and quality control. *J. Agric. Food Chem.*, 57(16): 7395-7400.

\*\*\*\*\*